supporting the general importance of the ETP moiety for HKMT inhibition[8], rather than a unique structural feature of chaetocin. In our view, any 'specificity' for SU(VAR)3-9 is most likely due to the increased sensitivity of this particular enzyme (versus other HKMTs) to thiol-reactive compounds.

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Competing financial interests

The authors declare no competing financial interests.

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Greiner *et al.* reply—in our paper[1], we presented several lines of evidence that chaetocin functions as specific inhibitor of SU(VAR)3-9. We had initially concluded that the disulfide warhead was not essential for chaetocin's inhibitory capacity on the basis of our observation of substantial inhibition of *Drosophila melanogaster* SU(VAR)3-9 by chaetocin, even in the presence of high concentrations of DTT1. Cherblanc *et al.*[2] now revisit this issue in the context of SUV39H1, the human ortholog of SU(VAR)3-9.

We note that Cherblanc *et al.*[2] find a IC_{50} for hSUV39H1 similar to that observed in our initial experiments. However, the availability of new compounds has enabled them to directly test our initial hypothesis. Their use of **2**, lacking chaetocin's disulfides, indeed suggests that, in contrast to our initial hypothesis, the disulfide bond within chaetocin has a substantial role. However, it is interesting that a structurally divergent ETP model compound (**3**) containing the disulfide bond has a 30-fold higher IC_{50}. This clearly suggests that chaetocin does not solely inhibit *Drosophila* SU(VAR)3-9 activity through the presence of its disulfide bonds (which **3** has as well) but also direct binding to the enzyme.

There is an apparent discrepancy between our data and the data of Cherblanc *et al.*[2] with regards to the mechanism of inhibition that may be due to differences in assay conditions: we stopped the methyltransferase reaction after 5 min (as opposed to 1.5 h for Cherblanc *et al.*[2]) and did not preincubate the enzyme with the inhibitor. By doing this, we excluded the effect that chaetocin's disulfide bonds might have on the enzyme after an extended incubation and could observe competitive binding of SAM to SU(VAR)3-9. On the basis of our findings and the new data of Cherblanc *et al.*[2],we assume that chaetocin binds the enzyme competitively and subsequently reacts with critical residues, therefore acting as a suicide inhibitor. It will be interesting to see this tested in a structural analysis. This mechanism would argue against the suggestion from Cherblanc *et al.*[2] that SU(VAR)3-9's susceptibility to the compound is solely based on the difference to reactive thiols, a hypothesis that can and should now be tested in a rigorous manner.

It is also worth noting that we were aware of the potential for nonspecific effects in our original work and had to perform a careful titration of chaetocin to prevent its global effect on cell physiology (we used SL2 *Drosophila* cells), establishing a narrow concentration window that enabled us to analyze the effects of chaetocin on histone modification separately from its induction of apoptosis. As we only detected changes in H3K9 methylation and not at any other methylation site either by MS or by western blotting, we concluded that chaetocin specifically targets the class of enzymes known to methylate this residue (for example, G9a and SU(VAR)3-9). We therefore think that chaetocin, at least in this narrow concentration window, can be used to modulate H3K9me *in vivo* and is thus a useful compound for further investigations.

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